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4 authors, including:

Amit R. Chavan
Vidya Pratishthan's, College of Engineering, Ba…

Rajveer Shastri
Vidya Pratishthan's, Kamalnayan Bajaj Institut…

Deosarkar Shankar
Dr.Babasaheb Ambedkar Technological Univer…

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Counting of Frozen Semen Straws using Image Processing

Amit R. Chavan, A. R. Shastri, R. K. Shastri and S. B. Deosarkar
Department of Electronics and Telecommunication Engineering
VPCOE Baramati (University of Pune),
Baramati, India.
E-mail: amitrc7th@gmail.com, aparnadjoshi@yahoo.com, rajveer_shastri@yahoo.com, sbdeosarkar@yahoo.com

***Abstract*—The proposed work is to verify the count of the semen straws which are kept in a compact bunch by using image processing techniques. These straws are kept in liquid nitrogen for the straw content preservation purpose. Due to the liquid nitrogen characteristics and safety requirements for both semen straws as well as workers working in such an environment, a non-harming, rapid process for straw counting is required. So, straw counting using image processing algorithm is carried out. The counting problem is solved by using several image enhancement, image segmentation and object counting techniques. For the problem solving, many traditional methods like contrast stretching, thresholding, morphological processing as well as advanced methods like color based information removal and image enhancement using intensity factor are used.**

Keywords-Bulk Object Counting; Image Enhancement; Image Segmentation; Intensity Factor; Matrix Labeling; Minimum Area Estimation

I. INTRODUCTION

In this work, the necessary task is to verify the count of the straws which are kept in a compact bunch by using image processing techniques. These straws are kept in liquid nitrogen for preservation purpose, and due to the contents of straws, they must be kept under this liquid. Due to characteristics of liquid nitrogen and safety requirements for both semen straws as well as workers working in such an environment, a non-harming process for straw counting is required. So, straw counting using image processing is one of the best possible ways.

A. Problem Statement

This work is one of the basic proposed requirements of the BAIF (Bharatiya Agro-Industrial Foundation) Central Research Station, Urulikanchan, Dist. Pune. The main requirement of the Frozen Semen Lab, BAIF is to verify the count of semen straws after manual counting, via image processing techniques. The problem is: these straws must be kept under liquid nitrogen due to the biological contents of straws [1], [2].

Liquid nitrogen (LN_2) is an extremely cold liquid. Its boiling point is −195.9 °C, due to which it evaporates at room temperature rapidly [1]-[3]. Exposure of LN_2 to an oxygen deficient atmosphere may cause dizziness, drowsiness, nausea, vomiting, excess salivation, diminished mental alertness, loss of consciousness and death. Physical contact with liquid or cold vapors can cause severe frostbite [3].

Currently, in the lab, the process of straw counting is totally manual. In this, the worker places a straw-filled container (with an unknown count), and an empty container are kept together, in a thermocol container, having LN_2 in it. The worker picks up one or two straws in a fork, counts it in mind and rapidly puts in the empty container. The workers require too much concentration in this process for keeping accuracy, and he cannot even speak or respond to others during the task. By the fastest speed, a skillful worker at BAIF requires minimum 8.5 minutes to count 450 straws and approximately 13.5 minutes to count 750 straws. But still, a doubt about accuracy rises. After counting, the straws are kept in containers having LN_2 in it. The counted straws then delivered to doctors, farmers or other research centers [1].

II. FRAMEWORK

In this proposed work, the traditional framework is followed as shown in Fig. 1.

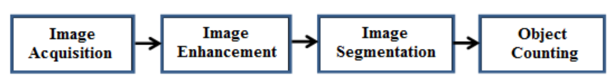

Figure 1. Generalized framework for object counting techniques using image processing

A. Image Acquisition

For image acquisition, here several different digital cameras are used, with different resolutions and features. Generally, cameras with higher resolution and higher numbers of CCDs are useful for this task [4]. These cameras are beneficial to achieve high range of data.

B. Image Enhancement

For enhancing the quality of images, the necessity tasks are noise removal [5]-[7], intensity slicing and contrast stretching [8] are important for images which are taken in the liquid like water or liquid nitrogen [9]. Top Hat Filtering is also used for image enhancement and better segmentation results [10].

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C. *Image Segmentation*

It is the set tradition in the image processing area that: for a successful object counting using image processing, someone cannot avoid the fact called as image segmentation. Since 2001, image segmentation is often used in most of the processes which requires object counting using image processing [11]. The objects may have irregular or regular shapes, in a compact group or may be at a distinguishable and separable distance. Morphological Operations, Background estimation and removal these are some methods to be performed in this process [11]-[14]. Thresholding is the soul part of segmentation. There are several segmentation methods which are based on color information of image [15].

D. *Object Counting*

The segmented image is useful for object counting [11], [14], [16]. We can apply simple logic of object counting by area estimation. Also, several statistical methods are available for image counting [16]. Connected Component Algorithm is also preferred for this task [10].

III. METHODOLOGY

In this work, the proposed workflow is – to acquire the top view image of the frozen semen straw container (placed in LN_2), enhance the acquired image, perform image segmentation and object counting via area estimation or any other technique. Image related operations are tested in MATLAB® Simulator Software.

A. *Generation of Database*

By the courtesy of Frozen Semen Laboratory, BAIF, the image database of several frozen semen straw containers having different straw counts and straw arrangement patterns is generated. Total 70 images of top views of straw containers placed in LN_2 are taken by using several cameras with different resolutions. From this, the cameras with resolution 1600×1200 or higher generate best pictures for the task.

B. *Experimental Procedure*

A high resolution digital camera is used to take the top views of the containers placed in LN_2. Fig. 2 shows a specimen image of a top view of a container placed in LN_2, having 425 yellow colored straws.

Since colors of straws are different, it is necessary to predict color of straws placed in container, for better enhancement results. So for that, R, G and B components of that image are separated. Then, calculating the intensity factors [17] of each component, the contrast stretching [8] is applied to each color component plane. The formula used for intensity factor is,

$$\text{Intensity Factor} = \{(\Sigma\, I(x,y)127)/\Sigma\, I(x,y)\} \times 100. \quad (1)$$

Where $\Sigma\, I(x,y)_{127}$ is the sum of pixel values having intensity greater than L/2 – 1 (here 127) and $\Sigma\, I(x,y)$ sum of all pixel intensities.

The enhancement results and correspondent intensity (I) factors are shown and compared in Fig. 3.

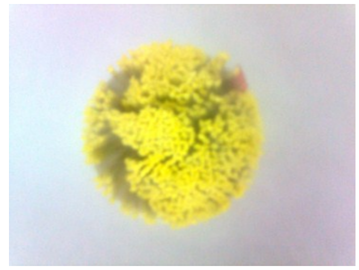

Figure 2. Original Image.

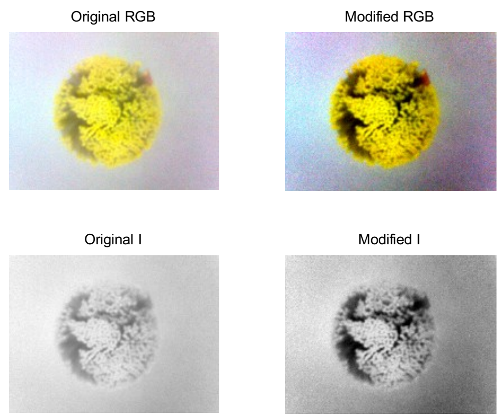

Figure 3. Comparison of Original and Enhanced Images.

Then, as per standard protocols, the removal of non-uniform illumination in the image background is necessary. This can be done by performing a morphological operation: Top Hat Filtering (THF). An important use of THF is in correcting the effects of non-uniform illumination. It performs morphological opening on grayscale or binary image with structuring element.

$$\text{Top Hat Filtering} = \text{Image} - \text{Opening of Image}. \quad (2)$$

For non-uniform background elimination, if we directly apply here THF [10] on the modified intensity image shown in Fig. 3, the results are not satisfactory as shown in Fig. 4. Such images cannot be treated as input for segmentation. So for a change, this enhanced image is applied to the background removal algorithm, in which all the light cyan and sky blue colored background pixels are removed. In this algorithm, the comparison of R, G, and B values of each position (pixel) is done (via simple subtraction) to estimate the undesired shades which are removed later. The result is shown in Fig. 5.

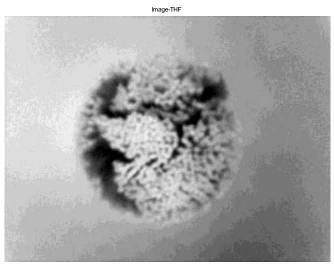

Figure 4. Result of THF subtraction performed on Enhanced Grayscale Image.

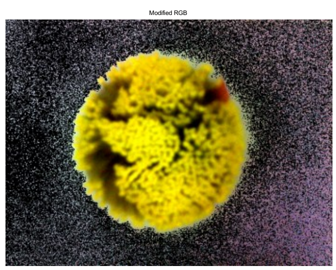

Figure 5. Color Based Background Segmentation of Enhanced Image.

Then, grayscale image from this processed image is obtained by (3):

$$\text{Grayscale} = (0.2989\times R) + (0.5870\times G) + (0.1140\times B) \quad (3)$$

The resultant grayscale image is shown in Fig. 6. Then, this image is directly applied to global thresholding, after Top Hat Filtering. The THF output is subtracted from the image shown in Fig. 6 to obtain output image with uniform background. The result is shown in Fig. 7. This obtained figure is now binarized using global thresholding [6]. The resultant image is shown in Fig. 8. The segmented image is then processed further for object counting, after the two rounds of morphological opening [6] by using disk of radius 5 pixels as a structuring element. Opening is done for eliminating the smallest areas which do not contain straw tips. The result is shown in Fig. 9. Now, this image is processed further for counting. Count is obtained by combining two ways: by ML4 (Matrix Labeling using 4-Directivity) and MAE DCM (Minimum Area Estimation and Division-and-Count Mechanism). In ML4, different label (number) is assigned to the each connected white area of the image [8]. This gives the number of connected objects found in image which represents the number of straw clusters in image. Fig. 10 shows the pseudo colored ML4 result, in which total 13 areas are detected.

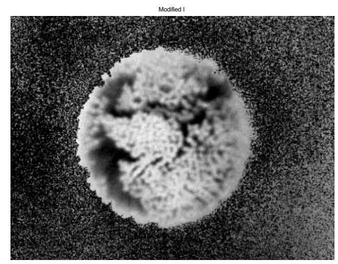

Figure 6. Grayscale of Color Segmented Image.

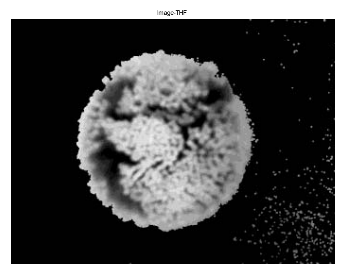

Figure 7. Resultant Grayscale Image after THF Subtraction.

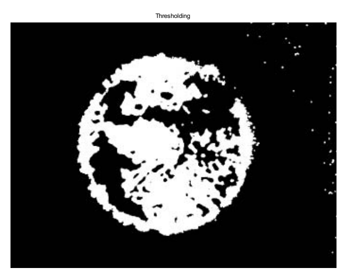

Figure 8. Global Thresholding Result.

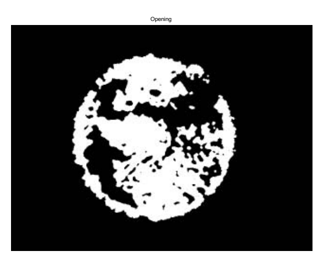

Figure 9. Result after Two Openings.

Figure 10. ML4 Result.

Now from these labeled areas, the straw area for this image is estimated by a Minimum Area Estimation (MAE) algorithm, which can be elaborated as:

$$\begin{aligned} &Area_s = \Sigma_{i,j}\ Pixel_{ij}(Area_s); \\ &\text{if } (Area_s > Area_n\ \&\&\ Area_s < Area_b\ \&\&\ Area_s < Area_d) \\ &\qquad Area_d = Area_s; \end{aligned} \tag{4}$$

Where, $Area_s$ is the currently selected area, $Area_n$ is the minimum area considered for the straw tips, $Area_b$ is the maximum area considered for the straw tips and $Area_d$ is the default desired estimated area of the straw tips for the image. The count is obtained by dividing the total white area by the desired estimated area. The MAE DCM result is shown in Fig. 11, in which the selected area is highlighted by red color and the count obtained is 404 straws.

Figure 11. MAE DCM Result.

IV. ANALYSIS OF RESULTS

The proposed algorithm can be applied for any colored straw except black. The drawback of the segmented images are we cannot purely separate one straw tip from another, since most of them are too close to separate. So, counting via area estimation method is must in such conditions. But another problem is, if the image is do not get properly segmented, then it can tend to data loss as seen by comparing Fig. 6 and Fig. 7. Data loss tends to inaccuracy in count. So improvement in segmentation method is necessary. Currently, this system counts straw tips within maximum 50 seconds, i.e. it saves almost 7.5 minutes of labor time. This is a good achievement.

V. CONCLUSION AND FUTURE SCOPE

The proposed algorithm proves very effective for counting of frozen semen straws kept in LN_2, which involves simple operations like contrast stretching, color removal, Top Hat Filtering, thresholding, opening and counting via area estimation. The method is simple and efficient, reduces time wastage, product wastage in manual straw counting procedure as well as possible health hazards by LN_2. This also increases performance and economy benefits. It is not too much sensitive to the non-uniform illumination, but sensitive to intensity and improper straw arrangement. If all straw tips are not having same intensity range, then data loss occurs due to the loss of straw tips in segmentation, resulting in inaccurate counting results. Using image processing techniques we can find the number of straws present in an image, even if they are placed together very compactly. This reduces the counting time significantly. Accuracy of results depends upon size of objects, whether or not any object are touching (in such case they might be labeled as one object), accuracy of background removal and the connectivity selected.

The present work can be extended by implementing a dedicated hardware to count the straws placed in LN_2 using programmable logic devices such as FPGA which can give rise to higher speed of computation. Making a cellphone application of the algorithm will be more convenient way.

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